PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Fatty Liver Index increase as a predictor of increased risk of cardiometabolic disease - Finding from the Kuopio Ischemic Heart Disease Risk Factor Study Cohort
AUTHORS	Olubamwo, Olubunmi; Virtanen, Jyrki K.; Pihlajamaki, Jussi; Mantyselka, Pekka; Tuomainen, Tomi-Pekka

VERSION 1 – REVIEW

REVIEWER	Amedeo Lonardo Azienda Ospedaliero-Universitaria Modena, Italy
REVIEW RETURNED	10-May-2019

GENERAL COMMENTS	GENERAL COMMENT In their prospective population-based study conducted on 501 men (out of a larger sample of 1289 individuals) with fatty liver disease (FLD) followed-up for 4 years, Olubamwo and Colleagues found that any increases in FLI, (a surrogate index of steatosis which is based on BMI, waist circumference, triglycerides and GGT) parallels similar increases of cardiometabolic risk (defined based on either cardiovascular disease or T2D or both). The manuscript may be improved through stylistic editing. In addition, a certain degree of ambiguity (ALD vs. NAFLD) should be resolved at the outset of the manuscript. Similarly, the contention that the larger the amount of intrahepatic fat content, the higher the cardiometabolic risk is not well documented by the present submission that the ability of FLI to capture the amount of steatosis is poor. Finally, References are imprecise, almost invariably refer to NAFLD and, also, must be updated. SPECIFIC COMMENT MAJOR The manuscript is not particularly reader-friendly. I would suggest avoiding long sentences and having the manuscript edited by a British native for conciseness and fluency. Page 4, lines 6-13 are an example of a quite long sentence which should be split and edited. Moreover sentences such as page 7 lines 50-57 may be reworded without any repetitions e.g. “In agreement with Bedogni, FLI was categorized as Low, Intermediate and moderate-high….” It is worth highlighting that “FLD” is indeed a composite disease entity which, further to HCV infection (of negligible prevalence in Finland), essentially includes both alcoholic and nonalcoholic fatty liver disease without any distinction. Although some Authors argue that this distinction is of dubious significance, as a matter of fact, our currently available information is strongly based on this distinction and others highlight the differences rather than the commonalities between the two. On this background, it may be argued that references almost invariably refer to NAFLD as

	opposed to alcoholic fatty liver disease (ALD). For example, Lines 18 -23 in page 1 refer to FLD in general but references are specific to NAFLD. Page 1 Lines 28 – 35 I am not sure that References 6 & 7 are well taken and I would suggest reworking this section on the natural history of NAFLD based on current paradigms. For example, the study cited at Ref. 6 (now published in Eur J Gastroenterol Hepatol. 2019;31:224-229) is a very very small-sampled paired biopsy study conducted in as few as 10 NASH patients and I suggest simply deleting it. Rather, I would encourage these Authors in reporting theories highlighting the importance of the chemical structure of lipids stored in the liver rather than their quantity, although also the quantity may have some importance, especially as far as the cardiometabolic riskis concerned (Gastroenterology. 2018 Aug;155(2):282-302.e8. Nat Rev Gastroenterol Hepatol. 2015 Mar;12(3):126-7). However, conflicting with these “steato-centric” theories, more recent studies emphasize the role of the more advanced forms of NAFLD (i.e. the role of liver fibrosis) both in the “hepatic” and in the “extra-hepatic” natural history of NAFLD (Gastroenterology. 2018;155:443-457.e17). In addition, there are two seminal articles on cardiometabolic risk in NAFLD which cannot be omitted citing and discussing (J Gastroenterol Hepatol. 2016;31:936-44. J Hepatol. 2016;65:589-600). Page 5 line 11 “Liver biopsy is risky” - It is invasive, not particularly risky in experienced hands Page 5 “determine whether progression of FLD, as assessed by significant increase in FLI (over a four-year period), is associated with increased risk of future CMD when compared with stable disease.” It would be great if the Authors might be willing to declare what they expected to find and – based on previous findings (J Gastroenterol Hepatol. 2016;31:936-44. J Hepatol. 2016;65:589-600) – why. Page 22 – lines 43-48 . Based on the literature I have cited above, this statement should be softened. Page 23 “It is known that several mechanisms can be involved in atherosclerosis acceleration” This is an incomplete view. A recent article proposes a theory accounting for two different evolutions, a short pathway and an accelerated route to clinical events (which involve plaque rupture/thrombosis). It is possible that access to these different pathogenic pathways mirrors liver histology but data support more a role for inflammatory and fibrosis changes rather than the amount of intra-hepatic fat content [J Hepatol. 2018;68(2):335-352]. This alternative theory must be discussed and either confirmed or confuted based on findings reported here. In doing so, these Authors may be willing to discuss the paper by Fedchuk (Aliment Pharmacol Ther. 2014;40(10):1209-22) who found that the ability of FLI to quantify steatosis was poor: this test was not able to distinguish moderate from severe steatosis. Along the same line, Professor Targher’s group reported that “Patients with more "severe" NAFLD were more likely to develop incident diabetes; this risk increased across the ultrasonographic scores of steatosis (n = 3 studies), but it appeared to be even greater among NAFLD patients with advanced high NAFLD fibrosis score” (Diabetes Care. 2018;41:372-382). Again, this study puts fibrosis (not steatosis) in the spotlight as a risk factor for

	forthcoming metabolic complications associated with NAFLD and must be extensively discussed. Page 23 Line 54 “fibrosis and inflammation are important confounders of the relation between liver fat and FLI” sentence unclear. Please reword more clearly. Alternatively, delete it and discuss accurately the paper by Fedchuk and Nascimbeni. In this Referee’s opinion there is another major limitation of this submission which must be fairly acknowledged and commented. “Liver biopsy is risky and magnetic resonance imaging (MRI) is expensive”. These are not the only two available techniques, luckily we have ultrasonography ! This is universally considered a first-line imaging technique to assess NAFLD both in clinical practice and in the setting of epidemiological studies; to rule out focal liver lesions; moreover, semi-quantitative indices can also provide informative indications regarding metabolic derangements and liver histology (World J Gastroenterol. 2018;24:3361-3373; BMJ. 2018 Jul 12;362:k2734; Metabolism. 2017 Jul;72:57-65). In addition, fibroscan can accurately asses steatosis and liver stiffness so more precisely depicting (than FLI does) liver histology per non-invasive route in epidemiological settings (PLoS One. 2018;13(9):e0200656. Hepatology. 2018;67:134-144. Gut. 2016 ;65:1359-68). This study has many tables and no graphs. Do these Authors believe that they could transform into graphs-histograms-visual illustrations any of their innumerable numerical data ? MINOR Throughout the manuscript: capitalize GGT (not ggt) Page 7, line 20 “Metabolic syndrome status was defined according to the harmonized criteria” Readers would appreciate a short summary/recall of such criteria. Page 8 Line 15 “non-patient research facility”-> ? Page 22 “The insulin resistant fatty liver then overproduces glucose and VLDL.” A bibliographic reference is requested

REVIEWER	Stefano Azienda USL Modena
REVIEW RETURNED	30-May-2019

GENERAL COMMENTS	Fatty Liver Index increase as a predictor of increased risk of cardiometabolic disease - Finding from the Kuopio Ischemic Heart Disease Risk Factor Study Cohort The study is timely and interesting but some concerns may be raised. Major comments - The Authors arbitrarily choose to use a composite outcome summarizing T2D and CVD, namely CMD. This is debatable. The analysis should be also performed for separated outcomes. - CVD is not specified in methods section: coronary heart disease? stroke also included? Please specify.

	- Outcomes detection is largely undirected, based on registries and ICD-codes, rather than on a structured follow-up. This should be acknowledged. - Literature should be updated: o Recent metanalytical studies providing evidence of the association between NAFLD and incident T2D/MetS and CVD should be included. E.g. Ballestri S et al. J Gastroenterol Hepatol. 2016;31:936-44.; Targher et al. J Hepatol. 2016;65:589-600. o Some studies demonstrated that either reversal or improvement of NAFLD assessed by liver ultrasound will translate into either protection from or a decreased risk of developing incident T2D. E.g. Yamazaki H et al. Diabetes Care 2015; 38:1673–1679. o A recent meta-analysis has reported that more severe ultrasonographic NAFLD forms were associated with an increased risk of incident T2D (Mantovani A et al. Diabetes Care 2018). o Dated back 2014 a long-term follow-up Finnish study has reported that more severe ultrasound assessed NAFLD was independently associated with an increased risk of incident CVD (Pisto et al. BMJ 2014) Please discuss. Minor comments Reference 2-3 is duplicated.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
MAJOR
The manuscript is not particularly reader-friendly. I would suggest avoiding long sentences and having the manuscript edited by a British native for conciseness and fluency.

Response: The manuscript has been edited to improve the readability, avoiding long sentences and repetitions.

It is worth highlighting that “FLD” is indeed a composite disease entity which, further to HCV infection (of negligible prevalence in Finland), essentially includes both alcoholic and nonalcoholic fatty liver disease without any distinction. …
…. … On this background, it may be argued that references almost invariably refer to NAFLD as opposed to alcoholic fatty liver disease (ALD). For example, Lines 18 -23 in page 1 refer to FLD in general but references are specific to NAFLD.

Response: The fact that FLD is a composite disease has been reflected in the passage (Introduction. Page 4 lines 5-8). In addition, as much as it is available, we have cited references of both AFLD and NAFLD studies.

Page 1 Lines 28 – 35 I am not sure that References 6 & 7 are well taken and I would suggest reworking this section on the natural history of NAFLD based on current paradigms. For example, the study cited at Ref. 6 (now published in Eur J Gastroenterol Hepatol. 2019;31:224-229) is a very very small-sampled paired biopsy study conducted in as few as 10 NASH patients and I suggest simply deleting it. Rather, I would encourage these Authors in reporting theories highlighting the importance of the chemical structure of lipids stored in the liver rather than their quantity, although also the quantity may have some importance, especially as far as the cardiometabolic riskis concerned

(Gastroenterology. 2018 Aug;155(2):282-302.e8. Nat Rev Gastroenterol Hepatol. 2015 Mar;12(3):126-7). However, conflicting with these “steato-centric” theories, more recent studies emphasize the role of the more advanced forms of NAFLD (i.e. the role of liver fibrosis) both in the “hepatic” and in the “extra-hepatic” natural history of NAFLD (Gastroenterology. 2018;155:443-457.e17). In addition, there are two seminal articles on cardiometabolic risk in NAFLD which cannot be omitted citing and discussing (J Gastroenterol Hepatol. 2016;31:936-44. J Hepatol. 2016;65:589-600).

Response: We have rewritten the introduction section to include description of the natural history of NAFLD based on current paradigms. We also highlighted the importance of the chemical structure of lipids stored in the liver rather than their quantity. The two seminal articles on cardiometabolic risk in NAFLD, (J Gastroenterol Hepatol. 2016;31:936-44. J Hepatol. 2016;65:589-600), have also been cited (Page 4, lines 9-19).

Page 5 line 11 “Liver biopsy is risky” - It is invasive, not particularly risky in experienced hands

Response: The sentence has been edited to remove the word “risky”. See page 5 lines 6 -7.

Page 5 “determine whether progression of FLD, as assessed by significant increase in FLI (over a four-year period), is associated with increased risk of future CMD when compared with stable disease.” It would be great if the Authors might be willing to declare what they expected to find and – based on previous findings (J Gastroenterol Hepatol. 2016;31:936-44. J Hepatol. 2016;65:589-600) – why.

Response: We expect to find that, among subjects with similar baseline FLI, significant increase in FLI is associated with greater risk of incident CMD compared with stable FLI. This has been stated on page 5, lines 16-23.

Page 22 – lines 43-48 . Based on the literature I have cited above, this statement should be softened.
Response: The statement has been deleted.

Page 23 “It is known that several mechanisms can be involved in atherosclerosis acceleration” This is an incomplete view. A recent article proposes a theory accounting for two different evolutions, a short pathway and an accelerated route to clinical events (which involve plaque rupture/thrombosis). It is possible that access to these different pathogenic pathways mirrors liver histology but data support more a role for inflammatory and fibrosis changes rather than the amount of intra-hepatic fat content [J Hepatol. 2018;68(2):335-352]. This alternative theory must be discussed and either confirmed or confuted based on findings reported here. ……
…. Again, this study puts fibrosis (not steatosis) in the spotlight as a risk factor for forthcoming metabolic complications associated with NAFLD and must be extensively discussed.

Response: The theory on the accelerated pathway of atherosclerosis in FLD has been updated accordingly (page 23 lines 1-13).
Page 23 Line 54 “fibrosis and inflammation are important confounders of the relation between liver fat and FLI” sentence unclear. Please reword more clearly. Alternatively, delete it and discuss accurately the paper by Fedchuk and Nascimbeni.

Response: The sentence has been rewritten to convey more accurately what was meant.
“…However, since FLI also correlates with degree of NASH and degree of fibrosis, higher degrees of inflammation or fibrosis results in higher FLI among individuals with similar histological degrees of steatosis…..”. See page 24, lines 1-3.

In this Referee's opinion there is another major limitation of this submission which must be fairly acknowledged and commented. "Liver biopsy is risky and magnetic resonance imaging (MRI) is expensive". These are not the only two available techniques, luckily we have ultrasonography! This is universally considered a first-line imaging technique to assess NAFLD both in clinical practice and in the setting of epidemiological studies; to rule out focal liver lesions; moreover, semi-quantitative indices can also provide informative indications regarding metabolic derangements and liver histology (World J Gastroenterol. 2018;24:3361-3373; BMJ. 2018 Jul 12;362:k2734; Metabolism. 2017 Jul;72:57-65). In addition, fibroscan can accurately asses steatosis and liver stiffness so more precisely depicting (than FLI does) liver histology per non-invasive route in epidemiological settings (PLoS One. 2018;13(9):e0200656. Hepatology. 2018;67:134-144. Gut. 2016 ;65:1359-68).

Response: the possible role of FLI in identifying patients in need of further evaluation, including radiological examination, has been mentioned. Page 5, lines 9-11.

This study has many tables and no graphs. Do these Authors believe that they could transform into graphs-histograms-visual illustrations any of their innumerable numerical data?

Response: After much deliberation, it was concluded that the results of the statistical analyses are best presented as tables.

MINOR
Throughout the manuscript: capitalize GGT (not ggt)
Response: GGT has been capitalized throughout the manuscript.

Page 7, line 20 "Metabolic syndrome status was defined according to the harmonized criteria"
Readers would appreciate a short summary/recall of such… criteria.
Response: Further details on the harmonized criteria have been provided (page 7, lines 7-12).

Page 8 Line 15 "non-patient research facility"-> ?
Response: the statement has been reworded as "University affiliated research center". Page 9, line 10.

Page 22 "The insulin resistant fatty liver then overproduces glucose and VLDL." A bibliographic reference is requested.

Response: A bibliographic reference has been provided for the statement (Page 22, line 20, reference 42).

Reviewer: 2
MAJOR
The Authors arbitrarily choose to use a composite outcome summarizing T2D and CVD, namely CMD. This is debatable. The analysis should be also performed for separated outcomes.
Response: The analyses has been performed for CVD and T2D separately. The results are presented in Tables 1 and 2 in the appendix. The comments have been added to the relevant sections.
Methods - (Page 10, lines 7-8).
Results - (Page 12, line 25 to page 13 line 3).
Discussion - (Page 23, lines 16-19).

CVD is not specified in methods section: coronary heart disease? stroke also included? Please specify.

Response: The CVD components are cardiac and vascular diseases outlined in the Tenth International Classification of Diseases (ICD) …ICD 10 100 to 199. See (Page 8, line 22 to page 9, line 2).

Outcomes detection is largely undirected, based on registries and ICD-codes, rather than on a structured follow-up. This should be acknowledged.
Response: The limitation of the registry-based follow-up has been acknowledged. See (Page 24, line 16).

- Literature should be updated:

…..Recent metanalytical studies providing evidence of the association between NAFLD and incident T2D/MetS and CVD should be included. E.g. Ballestri S et al. J Gastroenterol Hepatol. 2016;31:936-44.; Targher et al. J Hepatol. 2016;65:589-600. ….
Response: We have updated our references in the introduction and discussion sections.

MINOR

Reference 2-3 is duplicated.

Response: The duplicated reference has been removed.

VERSION 2 – REVIEW

REVIEWER	Amedeo Lonardo Azenda Ospedaliero-Univesritaria of Modena, Italy
REVIEW RETURNED	11-Aug-2019

GENERAL COMMENTS	This submission is improved as a result of these Authors' compliance with the Reviewers' suggestions.